

CLAIMS

What is claimed is:

1. A method of identifying a compound that modulates a biological activity of a potassium channel, comprising:

- 5 (a) providing a structure comprising a potassium channel polypeptide and a KCR1 polypeptide;
- (b) contacting the test compound with the structure;
- (c) determining a biological activity of the potassium channel polypeptide in the presence of the test compound;
- 10 (d) comparing the biological activity of the potassium channel polypeptide in the presence of the test compound to the biological activity of the potassium channel polypeptide in an absence of the test compound, wherein a difference between the biological activity of the potassium channel in the absence of the test compound and the biological activity of the potassium channel polypeptide in the presence of test compound indicates modulation of a biological activity of the potassium channel.
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2. The method of claim 1, wherein the structure comprises a cell.

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3. The method of claim 2, wherein the cell is isolated from a subject.

4. The method of claim 1, wherein the structure comprises a lipid bilayer.

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5. The method of claim 1, wherein the structure is a cell that has been transfected with a nucleic acid encoding an exogenous KCR1 polypeptide

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6. The method of claim 1, wherein the structure is a cell that has been transfected with a nucleic acid encoding an exogenous potassium channel polypeptide.

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7. The method of claim 1, wherein the potassium channel is HERG.

8. The method of claim 7, wherein the HERG potassium channel is  
5 comprises a polypeptide sequence as set forth in SEQ ID NO: 3.

9. The method of claim 8, wherein a nucleic acid encoding the HERG potassium channel is heterologous.

10. The method of claim 8, wherein a nucleic acid encoding the  
10 HERG potassium channel is polycistronic.

11. The method of claim 1, wherein the KCR1 polypeptide is  
15 encoded by a nucleic acid comprising SEQ ID NO: 1.

12. The method of claim 11, wherein the nucleic acid is  
heterologous.

13. The method of claim 11, wherein the nucleic acid is  
20 polycistronic.

14. The method of claim 1, wherein the determining comprises  
employing a patch clamp apparatus.

15. The method of claim 1, wherein the biological activity of a  
25 structure comprising a potassium channel polypeptide and a KCR1  
polypeptide in the presence of a test compound is determined in the presence  
of an MiRP1 polypeptide.

16. The method of claim 1, wherein the structure further comprises a  
30 MiRP1 polypeptide.

17. The method of claim 16, wherein the MiRP1 polypeptide is  
encoded by a nucleic acid comprising SEQ ID NO: 4.

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18. The method of claim 17, wherein the nucleic acid is heterologous.

5 19. The method of claim 17, wherein the nucleic acid is polycistronic.

20. A method of predicting a propensity of a candidate drug to induce a cardiac arrhythmia, comprising:

- 10 (a) providing a structure comprising a potassium channel and a KCR1 polypeptide;
- (b) contacting a candidate drug with the structure;
- (c) determining a biological activity of the potassium channel in the presence of the candidate drug; and
- 15 (d) comparing the biological activity of the potassium channel in the presence of a KCR1 polypeptide and in an absence of a candidate drug to a biological activity of the potassium channel in the presence of the candidate drug, wherein a biological activity of the potassium channel in the presence of a candidate drug that is less than a biological activity of the potassium channel in an absence of the candidate drug is indicative of a propensity of the drug to induce cardiac arrhythmia.
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21. The method of claim 20, wherein the structure is selected from  
25 the group consisting of a cell and a lipid bilayer.

22. The method of claim 20, wherein the potassium channel is HERG.

30 23. The method of claim 22, wherein the HERG potassium channel comprises a polypeptide sequence as set forth in SEQ ID NO: 3.

24. The method of claim 23, wherein a nucleic acid encoding the HERG potassium channel is heterologous.

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25. The method of claim 23, wherein a nucleic acid encoding the HERG potassium channel is polycistronic.

5           26. The method of claim 20, wherein the KCR1 polypeptide is encoded by a nucleic acid comprising SEQ ID NO: 1.

          27. The method of claim 26, wherein the nucleic acid is heterologous.

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          28. The method of claim 26, wherein the nucleic acid is polycistronic.

          29. The method of claim 30, wherein the determining comprises employing a patch clamp apparatus.

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          30. The method of claim 20, wherein the structure further comprises a MiRP1 polypeptide.

20           31. The method of claim 30, wherein the MiRP1 polypeptide is encoded by a nucleic acid comprising SEQ ID NO: 4.

          32. The method of claim 31, wherein the nucleic acid is heterologous.

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          33. The method of claim 31, wherein the nucleic acid is polycistronic.

30           34. A method of identifying a candidate compound that modulates the biological activity of a complex comprising a HERG channel polypeptide and a KCR1 polypeptide, the method comprising:

- (a) placing a cell comprising a HERG channel polypeptide and a KCR1 polypeptide into a bathing solution;
- (b) determining an induced  $K^+$  current in the cell of step (a);

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- (c) adding a candidate drug to the bathing solution of step (a);
- (d) determining an induced  $K^+$  current in the cell of step (c); and
- (e) comparing the induced current of step (b) with the induced current of step (d), wherein the candidate compound modulates the biological activity of a complex comprising a HERG channel polypeptide and a KCR1 polypeptide if the current of step (d) is different from the current of step (b).

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35. The method of claim 34, wherein the HERG channel polypeptide comprises a polypeptide sequence as set forth in SEQ ID NO: 3.

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36. The method of claim 35, wherein a nucleic acid encoding the HERG potassium channel is heterologous.

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37. The method of claim 35, wherein a nucleic acid encoding the HERG potassium channel is polycistronic.

38. The method of claim 45, wherein the KCR1 polypeptide is encoded by a nucleic acid comprising SEQ ID NO: 1.

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39. The method of claim 38, wherein the nucleic acid is heterologous.

40. The method of claim 38, wherein the nucleic acid is polycistronic.

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41. The method of claim 34, wherein the determining comprises employing a patch clamp apparatus.

42. The method of claim 34, wherein the cell further comprises a MiRP1 polypeptide.

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43. The method of claim 42, wherein the MiRP1 polypeptide is encoded by a nucleic acid comprising SEQ ID NO: 4.

44. The method of claim 43, wherein the nucleic acid is heterologous.

5           45. The method of claim 43, wherein the nucleic acid is polycistronic.

46. The method of claim 34, wherein the cell is isolated from a subject.

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47. The method of claim 34, further comprising transfecting the cell with a nucleic acid sequence encoding a HERG channel polypeptide and a nucleic acid sequence encoding a KCR1 polypeptide.

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48. A modulator identified by the method of claim 34.

49. A method for identifying a candidate compound as a modulator of KCR1 expression, the method comprising:

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(a) contacting a eukaryotic cell sample with a predetermined concentration of the candidate compound to be tested, the cell sample comprising at least one cell comprising a DNA construct comprising in 5' to 3' order (i) a modulatable transcriptional regulatory sequence of a KCR1-encoding gene, (ii) a promoter of the KCR1-encoding gene, and (iii) a reporter gene which expresses a polypeptide capable of producing a detectable signal coupled to and under the control of the promoter, under conditions such that the candidate compound if capable of acting as a transcriptional modulator of the gene encoding the protein of interest, causes a measurable detectable signal to be produced by the polypeptide expressed by the reporter gene;

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(b) quantitatively determining the amount of the signal so produced; and

(c) comparing the amount so determined with the amount of produced signal detected in the absence of candidate compound

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being tested or upon contacting the cell sample with other compounds so as to thereby identify the candidate compound as a chemical which causes a change in the detectable signal produced by the polypeptide and which transcriptionally modulates expression of KCR1.

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50. The method of claim 49, which comprises separately contacting each of a plurality of identical cell samples with different candidate compounds, each cell sample containing a predefined number of identical cells under conditions wherein said contacting is effected with a predetermined concentration of each different candidate compound to be tested.

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51. A modulator identified by the method of claim 49.

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52. A method for identifying a candidate compound as a modulator of KCR1 expression, the method comprising:

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(a) contacting a eukaryotic cell sample with a predetermined concentration of the candidate compound to be tested, the cell sample comprising at least one cell comprising a DNA construct comprising in 5' to 3' order (i) a modulatable transcriptional regulatory sequence of a KCR1-encoding gene, (ii) a promoter of the KCR1-encoding gene, and (iii) a DNA sequence transcribable into mRNA coupled to and under the control of the promoter, under conditions such that the candidate compound if capable of acting as a transcriptional modulator of the KCR1-encoding gene, causes a measurable difference in the amount of mRNA transcribed from the DNA sequence;

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(b) quantitatively determining the amount of the mRNA so produced; and

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(c) comparing the amount so determined with the amount of mRNA detected in the absence of candidate compound being tested or upon contacting the cell sample with other compounds so as to thereby identify the candidate compound as a compound which

causes a change in the detectable mRNA amount and which transcriptionally modulates expression of KCR1.

53. The method of claim 52, which comprises separately contacting  
5 each of a plurality of identical cell samples with different candidate compounds, each cell sample containing a predefined number of identical cells under conditions wherein said contacting is effected with a predetermined concentration of each different candidate compound to be tested.

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54. A modulator identified by the method of claim 52.

55. A method for modulating potassium channel function in a subject, the method comprising:

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(a) administering to the subject an effective amount of a substance that provides expression of a KCR1-encoding nucleic acid molecule in a cell or tissue where modulated potassium channel function is desired; and

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(b) modulating potassium channel function in the subject through the administering of step (a).

56. The method of claim 55, wherein the subject is a mammal.

57. The method of claim 55, wherein the potassium channel function  
25 that is modulated in the subject comprises HERG function.

58. The method of claim 55, wherein the cell or tissue is a cardiac cell or tissue.

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59. The method of claim 55, wherein the administering is selected for the group consisting of intravenous administration, intrasynovial administration, transdermal administration, intramuscular administration, subcutaneous administration and oral administration.

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60. The method of claim 55, further comprising:
- (a) providing a gene therapy construct comprising a nucleotide sequence encoding a KCR1 polypeptide; and
  - (b) administering the gene therapy construct to a subject, whereby the function of a potassium channel in the subject is modulated.

61. The method of claim 60, wherein the KCR1 polypeptide is encoded by a nucleic acid comprising SEQ ID NO: 1.

62. The method of claim 60, further comprising administering the gene therapy vector to a cardiac cell or tissue in the subject.

63. A method for modulating potassium channel function in a subject, the method comprising:

- (a) preparing a composition comprising a modulator identified according to the method of claim 36, and a pharmaceutically acceptable carrier; and
- (b) administering an effective dose of the pharmaceutical composition to a subject, whereby potassium channel activity is modulated in the subject.

64. The method of claim 63, wherein the subject is a mammal.

65. The method of claim 63, wherein the potassium channel activity that is modulated in the subject comprises HERG activity.

66. A method of screening for susceptibility to a drug-induced cardiac arrhythmia in a subject, the method comprising:

- (a) obtaining a biological sample from the subject; and
- (b) detecting a polymorphism of a KCR1 gene in the biological sample from the subject, the presence of the polymorphism indicating the susceptibility of the subject to a drug-induced cardiac arrhythmia.

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67. The method of claim 66, wherein the biological sample comprises a nucleic acid sample.

68. The method of claim 67, wherein the polymorphism is an I447V  
5 polymorphism of the KCR1 gene.

69. The method of claim 68, wherein the polymorphism is detected  
by amplifying a target nucleic acid in the nucleic acid sample from the subject  
using an amplification technique.

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70. The method of claim 69, wherein the polymorphism is detected  
by amplifying a target nucleic acid in the nucleic acid sample from the subject  
using an oligonucleotide pair, wherein a first oligonucleotide of the pair  
hybridizes to a first portion of the KCR1 gene, wherein the first portion  
15 includes the polymorphism of the KCR1 gene, and wherein the second of the  
oligonucleotide pair hybridizes to a second portion of the KCR1 gene that is  
adjacent to the first portion.

71. The method of claim 70, wherein the first and the second oligo-  
20 nucleotides each further comprise a detectable label, and wherein the label of  
the first oligonucleotide is distinguishable from the label of the second  
oligonucleotide.

72. The method of claim 71, wherein said label of said first  
25 oligonucleotide is a radiolabel, and wherein said label of said second  
oligonucleotide is a biotin label.

73. The method of claim 67, wherein the polymorphism is detected  
by sequencing a target nucleic acid in the nucleic acid sample from the  
30 subject.

74. The method of claim 73, wherein the sequencing comprises  
dideoxy sequencing.

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75. The method of claim 67, wherein the step of detecting the polymorphism is detected by contacting a target nucleic acid in the nucleic acid sample from the subject with a reagent that detects the presence of the polymorphism and detecting the reagent.

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76. The method of claim 75, wherein the reagent comprises an allele specific oligonucleotide.

77. The method of claim 66, wherein the subject is a human subject.

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78. The method of claim 66, wherein the biological sample comprises a polypeptide sample

79. An oligonucleotide pair, wherein a first oligonucleotide of the pair hybridizes to a first portion of the KCR1 gene, wherein the first portion includes a polymorphism of the KCR1 gene, and wherein the second of the oligonucleotide pair hybridizes to a second portion of the KCR1 gene that is adjacent to the first portion.

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80. The oligonucleotide pair of claim 79, wherein the polymorphism is an I447V polymorphism of the KCR1 gene.

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81. The oligonucleotide pair of claim 79, wherein said first and said second oligonucleotides each further comprise a detectable label, and wherein said label of said first oligonucleotide is distinguishable from said label of said second oligonucleotide.

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82. The oligonucleotide pair of claim 81, wherein said label of said first oligonucleotide is a radiolabel, and wherein said label of said second oligonucleotide is a biotin label.

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83. A set of oligonucleotide primers comprising an anti-sense primer and a sense primer, wherein said oligonucleotide primer set is suitable for

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amplifying a portion of the KCR1 gene, wherein the portion includes a polymorphism of the KCR1 gene.

5        84.    The oligonucleotide set of claim 83, wherein the polymorphism is an I447V polymorphism of the KCR1 gene.

85.    A kit for detecting a polymorphism in a KCR1 gene, the kit comprising:

- 10        (a)    a reagent for detecting the presence of a I447V polymorphism of the *KCR1* gene in a biological sample from the subject; and
- (b)    a container for the reagent.

15        86.    The kit of claim 85, wherein the polymorphism is an I447V polymorphism of the KCR1 gene.

87.    The kit of claim 86, further comprising a reagent for amplifying a nucleic acid molecule containing an I447V polymorphism of the KCR1 gene.

20        88.    The kit of claim 87, wherein the amplifying reagent comprises a polymerase enzyme suitable for use in a polymerase chain reaction and a pair of oligonucleotides.

25        89.    The kit of claim 88, wherein a first oligonucleotide of the pair of oligonucleotides hybridizes to a first portion of the KCR1 gene, wherein the first portion includes the I447V polymorphism of the KCR1 gene, and wherein the second of the oligonucleotide pair hybridizes to a second portion of the KCR1 gene that is adjacent to the first portion.

30        90.    The kit of claim 85, further comprising a reagent for extracting a nucleic acid sample from a biological sample obtained from a subject.

91.    An assay kit for detecting the presence of a polymorphism of a *KCR1* gene encoding a KCR1 polypeptide in a biological sample, the kit comprising a first container containing a first antibody capable of

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immunoreacting with a KCR1 subunit polypeptide encoding by a KCR1 gene comprising a polymorphism, wherein the first antibody is present in an amount sufficient to perform at least one assay.

5            92.    The assay kit of claim 91, wherein the polymorphism is an I447V polymorphism of the KCR1 gene.

            93.    The assay kit of claim 91, further comprising a second container containing a second antibody that immunoreacts with the first antibody

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            94.    The assay kit of claim 93, wherein the first antibody and the second antibody comprise monoclonal antibodies.

            95.    The assay kit of claim 93, wherein the first antibody is affixed to  
15 a solid support.

            96.    The assay kit of claim 93, wherein the first and second antibodies each comprise an indicator.

20            97.    The assay kit of claim 96, wherein the indicator is a radioactive label or an enzyme.

            98.    An assay kit for detecting the presence, in a biological sample, of an antibody immunoreactive with a KCR1 polypeptide encoding by a KCR1  
25 comprising a polymorphism, the kit comprising a first container containing a human KCR1 polypeptide encoded by a KCR1 gene comprising a polymorphism that immunoreacts with the antibody, with the polypeptide present in an amount sufficient to perform at least one assay.

30            99.    The assay kit of claim 98, wherein the polymorphism is an I447V polymorphism of the KCR1 gene.

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